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Isolation and Characterization of Rhizospheric Bacteria from *Vuralia turcica* Rhizospheric Soil

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ABSTRACT

Plant growth-promoting rhizobacteria are highly active in soil ecosystems for legumes due to their biotic activities. *Vuralia turcica* (Kit Tan, Vural & Kucukoduk) Uysal & Ertugrul is a Turkish endemic legume plant with potential value as ornamental and food crops. However, reports of plant growth-promoting rhizobacteria in *V. turcica* rhizosphere are lacking in the literature. The purpose of this study was the isolation and characterization of endophytic bacteria from *V. turcica* rhizospheric soil. Ten bacterial strains were isolated and identified by comparing the 16S rRNA and 16S-23S rRNA ITS region. 4 isolates belonged to *Bacillus megaterium*, 3 strains belonged to *Stenotrophomonas rhizophila*, 1 strain belonged to *Rhodococcus erythropolis*, 1 strain belonged to *Xanthomonas albilineans*. The remaining 1 strain belonged to *Lysobacter enzymogenes*, respectively.

Keywords: Growth-promoting, 16S rRNA, 16S-23S rRNA ITS region, Molecular characterization

1. Introduction

Plant growth-promoting rhizobacteria (PGPRs) can stimulate plant growth, colonize the rhizosphere, and thrive and compete with other soil microorganisms accounting for their plant growth-enhancing properties (Kloepper 1994). They can be subdivided according to their function into plant growth-friendly phytostimulators, plant-enhancing biofertilizers, organic waste degraders in soil, and biopesticides controlling fungal and microbial pathogens (Antoun & Prevost 2005). In addition, they can be divided into two groups in terms of their position to anchor and colonize: the first one, extracellular PGPR, colonizes the rhizosphere or unoccupied spaces between root cortex cells (Bhattacharyya & Jha 2012), and the second one, intracellular PGPR, where rhizobia occur in root cell nodular structures (Figueiredo et al. 2010; Bhattacharyya & Jha 2012).

Different genera of PGPR, such as *Bacillus*, *Enterobacter*, *Azospirullum*, *Pseudomonas*, and *Azotobacter*, improve the growth of chickpea (Roopa et al. 2012), tobacco (Zhang & Kong 2014), squash (Yildirim et al. 2006), lettuce (Barassi et al. 2006), and common bean (Barros et al. 2018), respectively. PGPRs are highly effective on legumes in soil ecosystems due to their biotic activities. In terms of application, they account for the increase in the yield of legumes by enhancing the concentration of mineral elements such as N, P, and K available in the soil.

In recent years, sudden climate changes have started to bring irreversible damages such as forest fires that are more common and affecting more expansive areas, the emergence of epidemics that threaten plant and animal species, the shift of biodiversity to the north to adapt to increasing temperatures, and the increase in drought and water scarcity due to decreased precipitation. However, it has been determined that there has been a decrease in agricultural lands per capita worldwide in recent years. It is stated that this decrease rate is 14.3% in developed countries, but it increases to 40% in developing countries (Aydinalp & Cresse 2008; Farooq et al. 2009). Therefore, the importance of PGPRs has been increasing in recent years. Some researchers have suggested that PGPRs have positive effects on plant development in many plant species by a series of mechanisms such as early germination, plant height, weight, development of shoot tissues, root growth, leaf area, early flowering, increase in chlorophyll amount, delay in the formation of the abscission layer in the leaf, and balancing the nutrient content (Sharafzadeh 2012; Singh et al. 2014; Fan et al. 2017). Plant growth-promoting rhizobacteria are becoming more widely used in agriculture, and they offer

an enticing alternative to chemical fertilizers, herbicides, and minerals. Also, excessive amounts of expensive fertilizers are used in intensive agricultural practices to produce optimum crop yields. Biological nitrogen fixation by symbiotic and nonsymbiotic bacteria, on the other hand, can help increase soil fertility and crop output while minimizing the use of chemical fertilizers. Legumes play a critical role in agricultural production because of their ability to fix nitrogen in conjunction with rhizobia.

Vuralia turcica (Tan et al. 1983) Uysal et al. (2014) is a Turkish endemic plant of legume that is potentially valuable to increase yield. However, reports of PGPRs in *V. turcica* rhizosphere are lacking in the literature. Therefore, this study aims to isolate and characterize PGPRs in rhizospheric soil where *V. turcica* grows.

2. Material and Methods

2.1. Soil and rhizome sampling

Four rhizospheric soil samples of approximately 100 g each and four rhizomes were collected from four *V. turcica* fields in April 2017 during the plant's blooming period. The fields were located close to the Eber and Akşehir lakes in Konya Province in Turkey. The coordinates of each sample collected are: L1 (Gölçayır, 38° 28′ 10.5′′ N/31° 21′ 04.4′′ E), L2 (Akşehir, 38° 28′ 17.328′′ N/31° 20′ 52.468′′ E), L3 (Dereçine, 38° 30′ 36.702′′ N/31° 17′ 56.702′′ E), L4 (Sultandağı, 38° 32′ 43.2168′′ N/31° 16′ 54.4728′′ E). A Global Positioning System (GPS; Magellan eXplorist 310) was used to determine the coordinates of the samples collected. Soil samples were obtained from the depth of rhizomes (~30 cm) and taken in sterilized ziplock containers, stored in an ice chest, and transported to the laboratory to use within 12h from the collection. Simultaneously, rhizome samples from the depth (~30 cm) were collected for mineral element analysis. The sample collection was implemented by the workers of Nezahat Gökyiğit Botanical Garden in Istanbul, Turkey.

2.2. Physicochemical properties of the rhizospheric soil samples

The collected soil samples were aseptically separated from rhizomes to determine the physicochemical properties of the soils. 5 g of air-dried soil was passed through a 2 mm mesh used for pH and electrical conductivity (EC) determination. Soil pH was measured in a 2.5:1 water/soil suspension using a pH meter (Hanna instruments-HI 2211) described by Jackson (1959). EC of each soil sample was determined using the method described by Richards (1954) using electrical conductivity (EC) meter (WTW series-inoLab-Cond-720) in a 5:1 distilled water: soil dilution.

2.3. Nutrient analysis of the rhizospheric soil samples and the collected rhizomes

The mineral element content of the collected soils and rhizomes was determined using the methods described by Tekdal et al. (2018). Rhizome samples were finely ground after drying at approximately 65°C for 48h. Soil samples were air-dried for two days and then separated through a 2 mm mesh for analysis. The ground rhizome and air-dried soil samples were used to determine mineral element content according to the methods described by Lindsay & Norvell (1978) and Olsen et al. (1954). Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Vista-Pro Axial, Varian Pty Ltd, Mulgrave, Australia) was used to determine the mineral concentrations of the samples. Certified standard reference materials obtained from the National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA) were used to calibrate the measurements.

2.4. Endophytic bacteria isolation

Endophytic bacteria were isolated from the collected soil samples. Modified solid Yeast Extract Mannitol (YEM) medium described by Vincent (1970) was used to screen PGPRs in the soil. 10 grams of the soil sample from the rhizosphere was diluted to 10−5–10−6 suspension using 100 mL sterilized water. The soil suspension of 100 µL was then spread over a petri dish (100 × 15 mm) containing a culture medium for PGPRs. The Petri dishes were left in the incubator for 48h at 28°C. Screened strains were transferred to a fresh YEM agar medium for bacteria, which were then stored at 4°C for future use. For the pre-selection of PGPRs, YEM agar medium containing either Bromothymol blue or Congo red was used. Gram staining of the isolates was also conducted.

2.5. Microbial identification system (MIS) analysis of isolated bacteria

Bacterial fatty acid methyl ester (FAME) analysis was carried out utilizing gas chromatography (6890N GC, Agilent Technologies INC., USA) and MIS tools (Sherlock 6.0 MIDI, Inc., Newark, DE, 2005).

2.6. DNA isolation and PCR analysis

DNA was extracted from approximately 200 mg of the sample using the commercially-available kit (QIAamp DNA Minikit (Cat#51304, Qiagen, Valencia, CA) following the manufactures' instructions. DNA quality was checked on 1.5% agarose gel and was quantified spectrophotometrically. It was then stored in TE buffer (10 mM Tris, 1 mM ethylenediaminetetraacetic acid, pH 8.0) at –20°C for use in PCR analysis. 16S rRNA and 16S-23S rRNA Internal Transcribed Spacer (ITS) primers of bacteria

were used for PCR amplification. 16S rRNA genes were amplified using the sense strand primer 5' AGAGTTTGATCCTGGCTCAG -3' (D1F) and the anti-sense strand primer 5' AAGGAGGTGATCCAGCC -3' (D1R). 16S-23S rDNA ITS region was identified using the sense strand primer 5' TGCGGCTGGATCCCCTCCTT -3' (FGPS1490-72) and the anti-sense strand primer 5' CCGGGTTTCCCCATTCGG -3' (FGPL132-38). The PCR mixture (25 µL) consisted of 5 ng of DNA, 0.125 U/µL Taq DNA polymerase (Fermentas), 0.2 mM dNTP mixture, 0.8 µM of forward and reverse primer each, the polymerase reaction buffer (1X). The PCR was performed as follows: 35 cycles of 94 °C for 7min, 94 °C for the 30s, 55 °C for 30s, 72 °C for 1min, and 72 °C for 5min. PCR products were sequenced by BM Laboratory Systems, Ankara, Turkey (https://www.bmlabosis.com).

The 16S sequences of the isolates L1S2, L1S3, L1S4, L2S1, L2S2, L2S3, L3S1, L4S1, L4S2, L4S3, and L4S4 were submitted to GenBank under accession numbers MT477132, MT477133, MT477134, MT477135, MT477136, MT477137, MT477138, MT477139, MT477140, MT477141, respectively whereas the GenBank accession numbers of 16S-23S rDNA ITS sequences of the isolates L1S2, L1S3, L1S4, L2S1, L2S2, L2S3, L3S1, L4S1, L4S2, L4S3, and L4S4 are MT460379, MT460380, MT460381, MT460382, MT460383, MT460385, MT460386, MT460387, MT460388, and MT460389, respectively.

2.7. Phylogenetic analysis

The sequencing results were analyzed using the NCBI database for homologous sequences of 16S rRNA and 16S-23S rRNA ITS region in GenBank at the National Center for Biotechnology Information (NCBI), Bethesda, USA, using the BLAST search program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul et al. 1990). The Tamura 3-parameter and the Kimura 2-parameter methods were used to calculate the differences in nucleotides (Kimura 1980; Tamura 1992). The neighbor-joining method (Saitou & Nei 1987) was used to build a phylogenetic tree by the Molecular Evolutionary Genetics Analysis (MEGA) program version 7.0 (https://www.megasoftware.net/). Aligned 16S rDNA and 16S-23S rDNA ITS sequences were evaluated with bootstrap analysis (1000 replicates) (Felsenstein 1985).

3. Results

3.1. pH, soil nutrient, and salt content determination

The first step of this work was soil sampling. In addition to the bacterial isolation, the soil samples were used as soon as possible to analyze the pH, salt composition, and nutrient components. The pH and salt composition of the samples are reported in Table 1. The results showed that all soil samples collected from four different points were alkaline, as the pH range of analyzed samples was from 7.50 to 8.20. The soil sample taken from location 2 (L2) had a high EC, 1654 µs/cm (Table 1).

Table 1- pH and salt levels of the soil samples

Depth (cm)	*Sample*	*Region*	*pH*	*Salt (µs/cm)*
0-30	L1	Gölçayır	7.50	3.18
0-30	L2	Akşehir	7.98	1654
0-30	L3	Dereçine	8.05	512
0-30	L4	Sultandağı	8.20	396

3.2. Determination of rhizome and soil nutrient components

The mineral element content of the rhizomes and soils collected was obtained by ICP-OES analysis, and the results are reported in Table 2. B, Mo, Cu, Fe, Mn, Ni, and Zn levels are much higher in rhizomes than in soil samples. In contrast, macronutrients are present at much lower levels in rhizomes as compared to the soil, while the reverse is true for N and C levels.

Table 2- The mineral nutrient content of the rhizome and soil samples of *V. turcica* collected from different natural habitats

*Samples**	*B*	*Mo*	*Cd*	*Cu*	*Fe*	*Mn*	*Ni*	*Zn*	*Ca*	*K*	*Mg*	*N*	*C*
	mg kg^{-1}								*%*				
	Rhizomes												
L1	28	1.11	0.04	11	404	16	0.52	30	0.33	0.72	0.37	1.29	47.39
L2	16	0.03	0.05	11	604	22	0.74	15	0.52	0.24	0.34	1.29	47.08
L3	27	0.49	0.04	15	380	52	0.67	33	0.38	0.53	0.18	1.72	46.72
L4	36	0.46	0.03	9	379	23	0.65	32	0.59	1.06	0.56	2.08	47.30
	Soils												
L1	4.22	0.05	0.01	1.43	8.71	0.05	0.05	0.05	616	24	92	0.37	7.99
L2	2.36	0.03	0.01	2.46	9.03	0.03	0.03	0.03	697	35	135	0.16	4.27
L3	0.54	0.01	0.02	3.99	7.79	0.01	0.01	0.01	253	12	53	0.06	4.07
L4	0.64	0.04	0.01	1.89	12.12	0.04	0.04	0.04	7038	10	1517	0.42	7.68

***:** The coordinates of each sample; L1: (Gölçayır, 38° 28′ 10.5′′ N/31° 21′ 04.4′′ E), L2: (Akşehir, 38° 28′ 17.328′′ N/31° 20′ 52.468′′ E), L3: (Dereçine, 38° 30′ 36.702′′ N/31° 17′ 56.702′′ E), L4: (Sultandağı, 38° 32′ 43.2168′′ N/31° 16′ 54.4728′′ E)

3.3. Pre-selection of isolated bacteria from samples of the soil from different environments of V. turcica

There were a total of 10 isolates. Among these isolates, 3 isolates were obtained from sample 1, 2 isolates were obtained from sample 2, 1 strain was obtained from sample 3, and 4 isolates were obtained from sample 4. Table 3 indicates the responses to red, bromothymol blue, and gram-staining. As a result of MIS analysis, various colonized bacteria are given in Table 4 on the rhizospheric soils of *V. turcica* were determined.

Table 3- Reactions of isolates to Congo red, Bromothymol blue and Gram-staining

Location	*Strain Code*	*Gram-staining*	*Congo red*	*Bromothymol blue*
1	L1S2	Negative-Coccus	Red	Orange-Basic
	L1S3	Negative-Coccus	Black	Fungus-Acidic
	L1S4	Positive-Coccus	Orange	Yellow-Orange-Basic
2	L2S1	Negative-Coccus	Red	Yellow-Orange-Basic
	L2S2	Negative- Coccus	no growth	Yellow-Basic
3	L3S1	Positive- Coccus	Red	Yellow-Green-Basic
4	L4S1	Negative- Coccus	Red	Yellow-Orange-Basic
	L4S2	Negative- Coccus	Red-Black	Yellow-Orange-Acidic
	L4S3	Negative-Bacillus	Red	Yellow-Orange-Acidic
	L4S4	Negative-Bacillus	Red	Yellow-Orange-Acidic

Table 4- MIS results of the bacteria isolated from soil samples

Location	*Strain Code*	*MIS result*
1	L1S2	*Kocuria kristinae*-GC subgroup A (0.692)
	L1S3	*Bacillus megaterium*-GC subgroup A (0.805)
	L1S4	*Virgibacillus pantothenticus* (0.675)
2	L2S1	*Bacillus megaterium*-GC subgroup A (0.792)
	L2S2	*Pseudomonas syringae*-syringae (0.380)
3	L3S1	*Stenotrophomonas maltophilia* (0.305)
4	L4S1	*Kocuria erythromyxa* (Deinococcus) (0.374)
	L4S2	*Bacillus viscosus* (0.496)
	L4S3	*Pseudomonas huttiensis* (0.605)
	L4S4	*Pseudomonas huttiensis* (0.552)

3.4. Phylogenetic analysis of soil-isolated bacteria

The 16S rRNA and 16S-23S ITS sequences of soil-isolated bacteria were compared to the 16S rRNA and 16S-23S ITS sequencing identified in the GenBank database and BLAST. The results of the 16S rRNA analysis showed that the 10 isolates could be attributed to five groups. Among them, 6 isolates were *Pseudomonas* sp., 1 isolate was *Arthrobacter* sp., and the other 3 were *Bacillus* sp., *Aurantimonas* sp., and *Sphingomonas* sp., respectively. One isolate was closely phylogenetically related to *Agrobacterium tumefaciens*, showing 100% similarity in their 16S rRNA sequencing. One strain was found to be closely phylogenetically related to *Bacillus megaterium* (Figure 1).

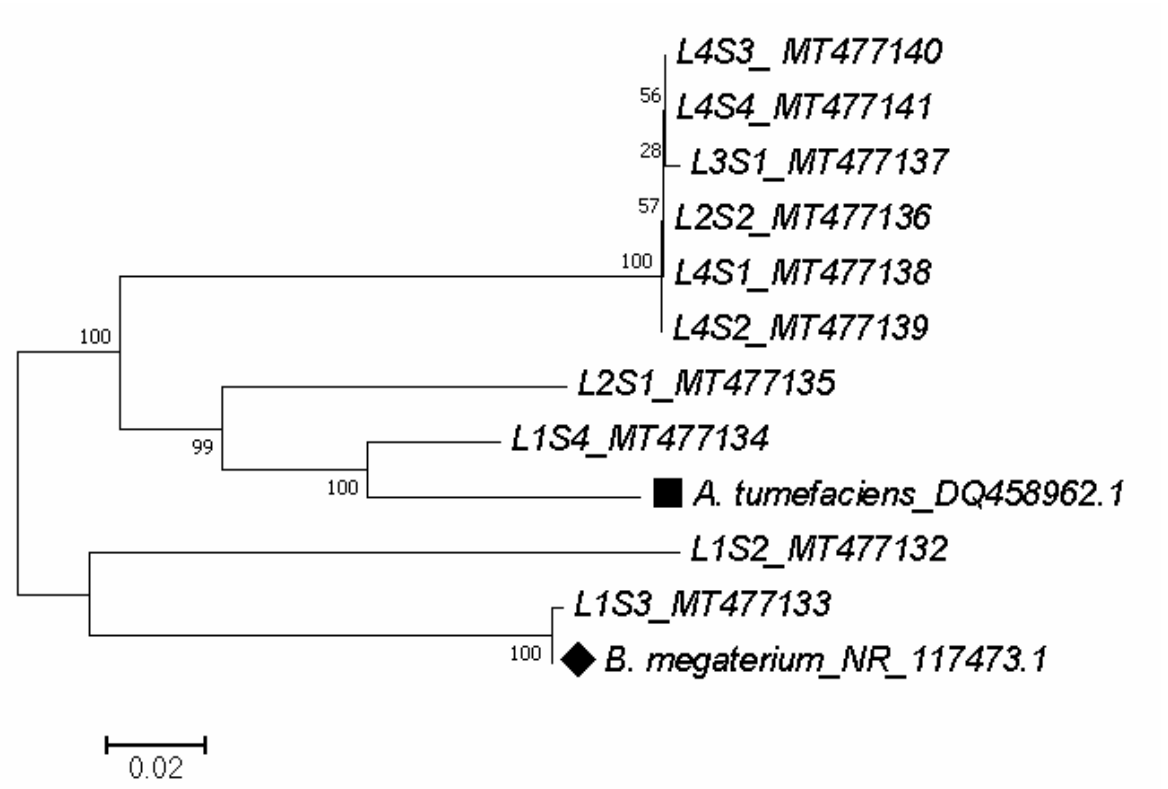

Figure 1- Neighbor-joining tree based on partial sequences of 16S rDNA region of the isolate for phylogenetic inference. As the outer group, DQ458962.1 and NR_117473.1 were chosen to depend on their 16S rRNA sequence similarities

On the other hand, the 16S-23S ITS analysis showed that the dominant genus was *Bacillus* in 6 isolates and *B. Megaterium* in the remaining 4 isolates. The strain L1S3 was also closely phylogenetically related to *B. megaterium* due to 16S-23S ITS analysis (Figure 2).

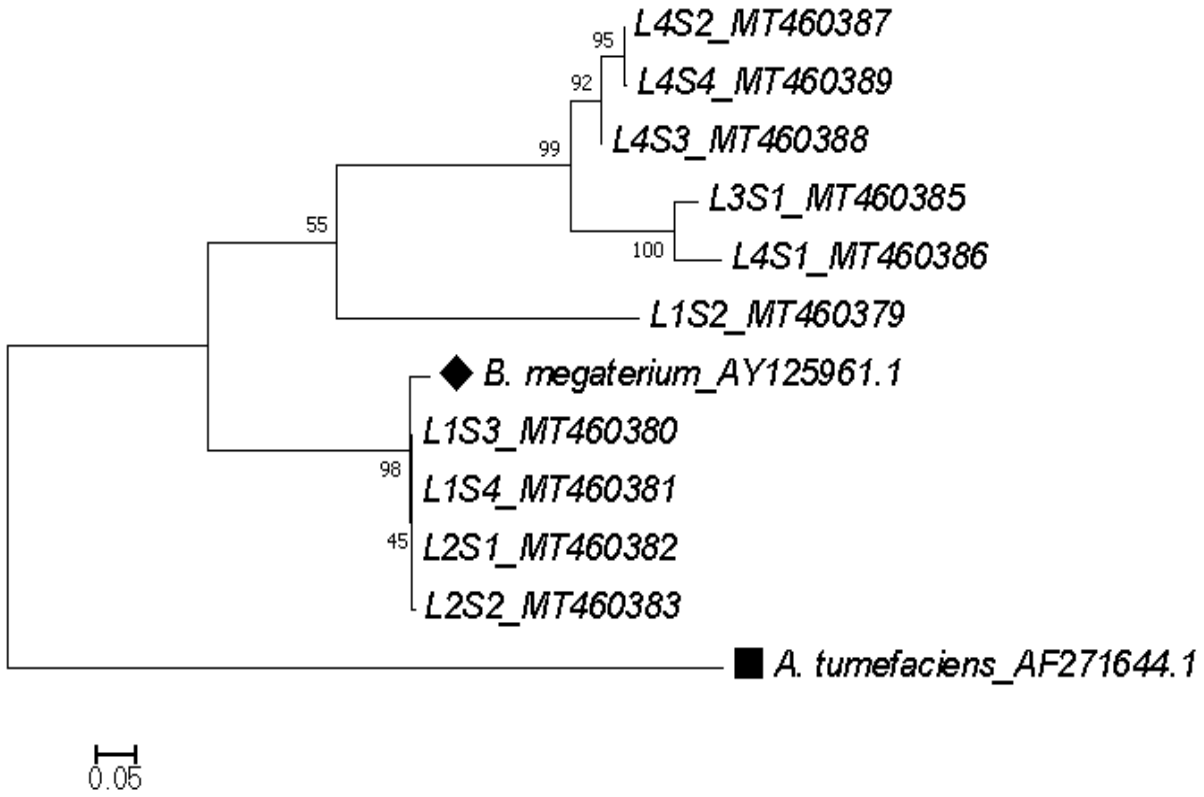

Figure 2- Neighbor-joining tree based on partial sequences of the ITS region of the isolate for phylogenetic inference. As the outer group, AF271644.1 and AY125961.1 were chosen to depend on their ITS sequence similarities

4. Discussion

The results of the pH and EC of collected soil samples, summarized in Table 1, indicate that all soil samples were alkaline.

Significant variations were found in the mineral analysis of rhizomes and soil samples. The quantities of minerals boron (B), copper (Cu), iron (Fe), zinc (Zn), and carbon (C) were elevated in rhizomes but in reduced concentrations in soil samples (Table 2). This discrepancy is most possibly due to the growth-promoting ability of bacteria present in *V. turcica* rhizospheres. In the phytoremediation of heavy metal-contaminated soils, *B. megaterium* is beneficial as this bacterium boosts the B desorption from the soil by enhancing its deposition in plants (Esringu et al. 2014). Also, *B. megaterium* can metabolize the Cu in the environment (Hohapatra et al. 1993). Therefore, high levels of Cu and B found in the rhizome samples are likely to be associated with *B. megaterium* activity.

Production and secretion of siderophores are an activity of *B. megaterium*, like most other PGPRs (Cornelis & Simon 2010). Increased secretion of siderophores by *B. megaterium* is reported in soils with iron deficiency (Arceneaux & Byers 1980). Siderophores can chelate ferric ions present in the environment into a siderophore-iron complex from which plants can acquire the Fe ion. The siderophore is then released into the rhizosphere and recycled (Santos et al. 2014). The amount of available iron found in *V. turcica* rhizomes while there is the facilitation of iron uptake can explain an iron scarcity in the soil through released siderophores of *B. megaterium*. Bacterial siderophores probably chelate the iron that is unavailable to *V. turcica* into plant-available forms.

In the literature, it is also mentioned that *B. megaterium*-related siderophores can ease Zn uptake for plants by solubilizing it (Kucey et al. 1989). Zn chelating behavior of *B. megaterium* is interpreted as the main reason for the high amount of Zn found in rhizomes in Zn deficient soil. Indirect growth-promotion pathways of *B. megaterium* include the activation of induced systemic resistance, the synthesis of antibiotics, the synthesis of siderophores, and the secretion of lytic enzymes (Ngoma et al. 2012). Besides providing iron ions to the host plants, *Bacillus spp*. produced siderophores also decrease the availability of the metal ions for competing species or pathogens present around the rhizosphere (Mathiyazhagan et al. 2004). Iron ions can easily be deprived of the rhizosphere because of the high affinity of siderophores with metal ions. This causes iron deficiency for surrounding pathogens and suppresses them (Kloepper et al. 1980). While protecting the host plant, this suppression enables *Bacillus spp*. to compete better for nutrients and gets more room to colonize (Labuschagne et al. 2010). The Fe ions were found in different compounds in the rhizomes than in the soil. With siderophores, *B. megaterium* competes better for Fe than pathogens and indirectly promotes plant growth through pathogen suppression.

The chlorophyll content of a plant represents the N-fixation activity and the plant's total N content (Kumawat et al. 2000). The photosynthetic capacity of a plant could be enhanced through *Bacillus sp.* N-fixing ability increases chlorophyll content. Thus, more photosynthates would be produced in the plant body to be further exuded from the roots (Elkoca et al. 2007). Rhizobacteria metabolize root exudates mostly for their carbon content (Vacheron et al. 2013). Regarding energy production, *B. megaterium* can metabolize more than 62 different carbon sources, including mono and disaccharides. This versatile carbon consumption-ability increases this species' survivability by facilitating colonization in different environments (Stülke & Hillen 2000; Vary et al. 2007).

Phytohormones induce and regulate plant growth, and PGPR are able to produce them. *Bacillus spp.* can directly influence root growth and shoot development by secreting auxins and cytokinins, which represent the main classes of plant growth-promoting compounds (Persello-Cartieaux et al. 2001; Arkhipova et al. 2005). In the literature, many reports show the ability of *B. megaterium* to produce plant hormones like auxins, gibberellins, cytokinins, and abscisic acid (Karadeniz et al. 2006). *B. megaterium* can induce shoot growth and ensure robust root development in plants through cytokine signaling (Ortiz-Castro et al. 2008). This bacterial species accounts for an increase in the root surface area of its host plant by supporting a robust root development, lateral root growth, root hair elongation, and root growth with an auxin/ethylene independent mechanism (López-Bucio et al. 2007). In this context, it is reasonable to assume that the growth of *V. turcica* is promoted by hormone-signaling pathways related to this bacterium as the soil is deficient in nutrients. Confirming this assumption requires a determination of the hormone levels and molecular interactions, including signaling pathways between the bacterium and the plant.

B. megaterium is a well-known PGPR species that promotes plant growth by N-fixation, P-solubilization, increased mineral uptake, and antimicrobial activity (López-Bucio et al. 2007). Many bacterial species were isolated from the soil samples taken around *V. turcica*'s natural habitat, but the most intriguing species were *B. megaterium*, *Rhodococcus erythropolis*, *Xanthomonas albilineans*, *Lysobacter enzymogenes*, and *Stenotrophomonas rhizophila* (Figure 1). *R. Erythropolis* is known to promote its host plant's growth in cold climate conditions (Trivedi et al. 2007). *X. albilineans* were reported to have pathogenic effects on sugarcane plants (Zhang et al. 2017). It is unclear whether this bacterium is pathogenic or not for *V. turcica*. However, even if it is, the antimicrobial activity of *B. megaterium* was probably useful in suppressing this bacterium, as healthy plant growth was naturally achieved. *B. megaterium* is an important bacterium frequently used in the industrial processing of fungicides, viral inhibitors, vitamins, and enzymes (Vary et al. 2007). It carries a high potential as a biofertilizer because of its plant growth-inducing abilities (Patel et al. 2016). It would be agriculturally and industrially interesting to collect more information on the molecular relations and secondary metabolite interactions between *B. megaterium* and the plant *V. turcica*, a resident of an

environmentally unfavorable place. Nutrient uptake for plants could be relieved through the secondary metabolites that PGPR secrete into the environment. *L. enzymogenes* exerts antifungal activity through lytic enzyme secretion (Jochum et al. 2006). This activity could account for the survival of *V. turcica* in its natural habitat by suppressing its fungal pathogens. *S. rhizophila was reported* to help plants to overcome salt stress enabling their growth in salty soils (Egamberdieva et al. 2016). Since the soil of *V. turcica*'s natural habitat is salty (Table 1), it could be supposed that *S. rhizophila* is helping *V. turcica* in relieving salt stress in its natural environment.

MIS is based on quantity as a percentage of fatty acids in microorganisms' cells (Şahin et al. 1999). Evaluation of MIS analysis is shown in Table 4. Moreover, the variety of isolated bacteria was different in soil samples at MIS analysis results. This could be due to the reduction in hydrocarbon sources in the soils. The number of FAME groups was high when the soils were collected, and then it started to decrease based on time. This study relies more on sequence analysis because of the narrow fatty acid library that is used in MIS analysis (1000 elements).

The role of isolated bacteria is unknown, so some speculations on the possible physiological roles of isolated bacteria were considered. Remarks on the estimation of the bacterial role should be proved experimentally.

5. Conclusions

With antibiotics potentially secreted, *B. megaterium* may restrict growth and increase the colonization rate of other microorganisms on the rhizome. Further analysis is needed to make certain conclusions on this subject, which reveals the antibiotics produced in the natural ecosystem of *V. turcica*. *B. megaterium* may be essential to *V. turcica*'s survival. As this plant's natural habitat is limited to an area, there is a lack of *B. megaterium* may be the cause of why *V. turcica* is not present elsewhere. The soil in which *V. turcica* naturally exists is also where the bacterium is found. By affecting the previous literature findings on this bacterial species, we strongly propose that *B. megaterium* plays a critical role in *V. turcica* plant growth and production in its natural environment. This is the first report of the presence of plant growth-promoting rhizobacteria in natural habitats and endemic plant *V. turcica* rhizospheric soils.

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